

Claims

1-6. (canceled)

7. (original) A packaging vector derived from HIV-2, comprising a 5' splice donor site, and an upstream and a downstream packaging signal sequence, wherein both the upstream and downstream packaging signal sequences are functionally deleted to substantially eliminate packaging of progeny viral RNA, but the splice donor site is functionally intact.

8. (original) The packaging vector of claim 7 wherein the deletions comprise no more than 164 nucleotides upstream of the SD and no more than 62 nucleotides downstream of the SD.

9. (original) The packaging vector of claim 7, wherein the deletions comprise nucleotides 306-458 upstream of the SD, and nucleotides 486-538 downstream of the SD; or
the deletions comprise nucleotides 306-370 upstream of the SD, and nucleotides 486-538 downstream of the SD; or
the deletions comprise nucleotides 371-458 upstream of the SD, and nucleotides 486-538 downstream of the SD.

10. (original) The packaging vector of claim 9 wherein the deletions comprise no more than 164 nt nucleotides upstream of the SD and no more than 62 nucleotides downstream of the SD.

11. (original) The packaging vector of claim 7 further comprising a 3'LTR, a 5'LTR, and a heterologous promotor CMV.

12. (original) The packaging vector of claim 10, wherein the 3'LTR is functionally deleted.

13. (original) The packaging vector of claim 12, wherein the 3'LTR is replaced with a heterologous transcriptional termination sequence.

14. (original) The packaging vector of claim 7, wherein the upstream packaging signal corresponds to nucleotides downstream from nucleotide 300 and upstream from the SD, and the downstream packaging signal corresponds to nucleotides downstream from the SD and upstream from nucleotide 539.

15. (original) The packaging vector of claim 7, wherein the functional deletions in the packaging vector decreases syncitia induction.

16. (original) An HIV packaging vector comprising a polynucleotide sequence which encodes HIV-2 proteins, wherein the polynucleotide sequence includes a mutation in a leader sequence upstream from a 5' splice donor site, and a mutation between the 5' splice donor site and an initiation codon followed by a stop codon of a gag gene, which results in HIV-2 RNA transcribed from the vector being substantially packaging defective.

17. (original) An HIV packaging vector comprising:

(a) a DNA segment from an HIV-2 genome, wherein the DNA segment comprises the HIV gag, pol and env genes; wherein the vector lacks an HIV-2 packaging sequence necessary to package HIV-2 RNA into virions, wherein the HIV-2 packaging sequence is a combination of a nucleotide sequence located between a 5' splice donor site and a nucleotide sequence located between the 5' splice donor site and an initiation codon of the gag gene on the HIV-2 genome;

(b) an intact 5' splice donor site; and

(c) a promoter operably linked to the DNA segment of (a), wherein the vector, when introduced into a eukaryotic host cell, expresses HIV-2 Gag, Pol, Rev, Tat, and Env proteins to form HIV-2 virions that are not packaged.

18-20. (canceled)

21. (currently amended) A cell that expresses or has been transfected with the packaging vector of [claims 7 or 17] claim 7.

22-27. (canceled)

28. (currently amended) A method for improving encapsidation of transgene RNA using retroviral packaging and transfer vectors, comprising in any order:

introducing into the target cell [the transfer vector of claim 18] an HIV-2 transfer vector comprising a polynucleotide sequence which encodes a transgene, and an HIV-2 packaging signal, 5' and 3' LTR in part or in whole, and promoter, but which does not encode one or more of a complete gag, pol or env gene, wherein the splice donor site is mutated to render it non-functional, which increases encapsidation of the transgene product, compared to encapsidation of the transgene product in the absence of the mutation in the splice donor site; and

introducing into the target cell the packaging vector of claim 16.

29. (original) The method of claim 28, wherein the packaging vector is an HIV-2 clone.
30. (original) The method of claim 29, wherein the packaging vector is an HIV-2 (ROD) clone.
31. (original) The method of claim 30, wherein the packaging vector is pROD(SD36).
32. (original) The method of claim 28, wherein the packaging vector is functionally and structurally divided into at least two packaging vectors.
33. (original) The method of claim 32, wherein the packaging vector is functionally and structurally divided between pROD(SD36/EM) and pCM-VSV-G or pCM-ENV(ROD).
34. (original) The method of claim 28, wherein the transfer vector is an HIV-2 clone.
35. (original) The method of claim 34, wherein the packaging vector is an HIV-2 (SGT) clone.
36. (original) The method of claim 34, wherein the transfer vector is pSGT-5(SDM).
37. (currently amended) The method of claim [26] 28, wherein the cell is a 293T cell.
38. (currently amended) [A] The method of claim 28, [for improving encapsidation of transgene RNA using retroviral packaging and transfer vectors, comprising, in any order:
introducing into the target cell the transfer vector of claim 18; and
introducing into the target cell the packaging vector of claim 16,]
wherein the packaging vector is an HIV-2(ROD) clone pROD(SD36), and the transfer vector is an HIV-2(SGT) clone pSGT-5(SDM), and the cell is a 293T cell.
39. (canceled)
40. (currently amended) The method of claim 38, wherein the packaging vector is functionally and structurally divided between the HIV-2(ROD) clone pROD(SD36/EM) and either pCM-ENV(ROD) or pCM-VSV-G, and the transfer vector is an HIV-2(SGT) clone pSGT-5(SDM)[, and the cell is a 293T cell].
- 41-42. (canceled)
43. (new) A cell that expresses or has been transfected with the packaging vector of claim 17.